Cell counting

This protocol is for suspension cells. If working with adherent cells, you should treat the culture with Trypsine as the first step (see: cell culturing protocol) and make only a 3x dilution of cells in Trypan Blue instead of the 10x adviced here.

Preparing sample

1. Transfer the cells into a Falcon tube.
2. Pellet the cells with the appropriate conditions (e.g. 300g 10')
3. Discard the supernatant and resuspend the pellet in 10 ml medium.
4. Take 20µl into an Eppendorf tube and mix it with 180µl Trypan Blue.
5. Put 10 µl in Bürker's chamber and put cover glass.

Counting cells in Burker's chamber

1. Put Bürker's chamber in the microscope. Focus on the grid lines of the chamber.
2. Count the live, unstained cells (alive cells doesn't reject Trypan Blue stain) in one set of 9 squares.
3. Include cells if they are inside the square or if they are on the boundary line of either the bottom or the right side of the square

Calculating number of viable cells/ml

The counted cells are the 10x dilution of 10ml total volume in 10 ul volume.
The cell number per mililiter is 10^4 times the number you've counted
As you had 10 ml, multiply it by 10.